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Correlation between development and increase of number of labial tooth rows in Ghost Frog tadpoles (Anura: Heleophrynidae)

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Abstract. The family Heleophrynidae is restricted to Southern Africa and comprises two genera with seven species. Tadpoles are well adapted, with huge sucker mouths, to live in the fast flowing headwaters of mountain streams. The unique sucker-like mouth has numerous transverse rows of labial teeth, which are used to attach themselves to the rock surface and to scrape algae from the rocks. In this paper data on the ontogenetic increase of labial tooth rows in tadpoles of four species of ghost frog are presented. Notes on the development of tadpoles and mouthparts are also presented.

Keywords. Feeding, algae, labial tooth rows, correlation, size.

Ghost frogs belong to the family Heleophrynidae, which consists of two genera: *Hadromophryne* Van Dijk 2008 and *Heleophryne* Sclater 1898, containing one and six species respectively (Du Preez and Carruthers, 2009). This anuran family is endemic to South Africa, Lesotho, and Swaziland (Boycott, 1999). This is an evolutionarily distinct family, their common ancestor and the remainder of all the neobatrachians having split from archaeobatrachians around the Jurassic period (± 160 MYA: Biju and Bossuyt, 2003) to form a sister group to all the other neobatrachians (San Mauro et al., 2005; Frost et al., 2006). Both adults and tadpoles are well adapted to live in the headwaters of mountain streams, tadpoles in particular possessing a huge sucker-like mouth and a dorso-laterally flattened body to overcome the fast flowing water (Fig. 1). This sucker-like mouth possesses numerous transverse rows of labial teeth, which are used to anchor the tadpole to the rock surface and for feeding. Tadpoles feed on algae growing on the surface of submerged rocks by scraping it off with the aid of numerous labial tooth rows (see Fig. 2; Rose, 1926; Boycott, 1972; Boycott and De Villiers, 1986). The cooler temperatures in these streams are responsible for an extended larval life stage, which can last up to 18-24 months (Boycott, 1988; Passmore and Carruthers, 1995; Channing, 2001; Du Preez and Carruthers, 2009). These highly specialised species have been trapped within the upper limits of specific catchments, thus leading to high vulnerability due to loss and degradation of natural habitat resulted from deforestation, fires, floods, erosion, damming and introduction of predatory fish. This has led to two species of Heleophrynidae having been classified as endangered (Boycott, 2004; IUCN, 2010). The situation is likely to be worsened by the effect of global warming on reduction in stream flow. The Table Mountain ghost frog (*Heleophryne rosei*) is considered Critically Endangered and Hewitt's ghost frog (*Heleophryne hewitti*) is rated Endangered, while the remaining species are currently considered Least Concern (IUCN, 2010).

Anuran tadpoles have very complex mouthparts. Especially the number, shape and size of teeth and their

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Fig. 1. Dorso-lateral photo of *Heleophryne hewitti* tadpole *in situ* (Witte River, Baviaanskloof, Eastern Cape, South Africa)

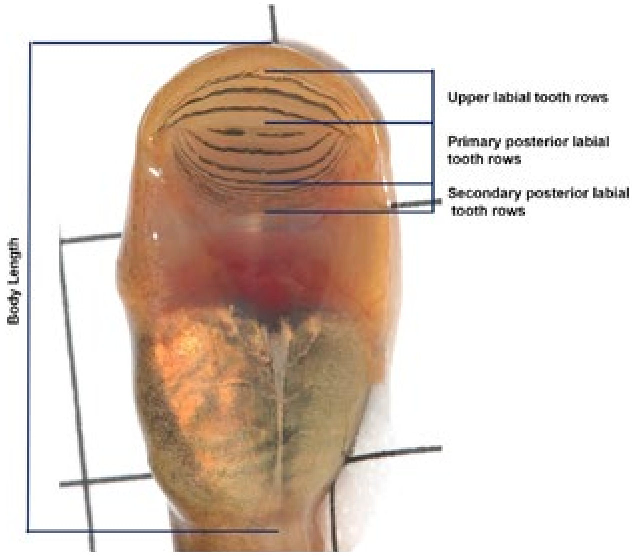

Fig. 2. Close-up view of *Heleophryne hewitti* tadpole showing the big sucker-like mouth and numerous transverse labial tooth rows (grid represents 1cm x 1cm)

arrangement in rows varies both interspecifically and ontogenetically within species (Altig and McDiarmid, 1999), which indicates an ecomorphological adaptation towards the pressures of the environmental conditions of the specific species, as well as to exploit different parts of the available food sources within an ecosystem (Khan and Mufti, 1994; Venesky et al., 2011). Labial teeth play a facultative role in feeding, functioning to anchor the oral disc to the substrate and, in conjunction with the jaw sheaths, to rake material off substrate (Venesky et al., 2010a,b, 2011). For example, many rows of labial teeth may be advantageous for taxa inhabiting fast-flowing streams where more teeth allow better anchorage to substrates to avoid being swept downstream (eg., *Hadromophryne* spp., *Heleophryne* spp., *Trichobatrachus robustus*, *Conraua* spp., *Petropedetes* spp.; Channing et al., 2013). Alternatively, fewer labial tooth rows may be advantageous for taxa living in lentic (standing water) habitats where they are not under selective pressures to anchor themselves firmly to a substrate (Venesky et al., 2010a,b). Despite the high morphological diversity found in tadpoles, they are often overlooked and understudied relative to other consumer groups in freshwater systems. There is also a lack of information on interspecific and ontogenetic changes in oral morphology, and how differences in tadpole mouthpart arrangements across species correlate with selective feeding (Altig et al., 2007).

All species of ghost frog tadpoles are characterised by a high number of keratinised labial tooth rows. The labial tooth row formula (LTRF) ranges from 4/14 to 4/17 (upper jaw/lower jaw) in each species, with the first row of posterior labial tooth rows usually divided (Hewitt, 1913; Rose, 1950; Wager, 1965; Van Dijk, 1966; Du Preez and Carruthers, 2009; Channing et al., 2012). We follow the use of LTRF laid out in Altig and McDiarmid (1999). *Hadromophryne natalensis* tadpoles differ from *Heleophryne* tadpoles by possessing a lower keratinised jaw sheath (Van Dijk, 2008; Du Preez and Carruthers, 2009; Channing et al., 2012). Visser (1971, 1985) described the development of individual fang-like teeth, which are replaced by rows of smaller teeth at 20-22 days in *Heleophryne* tadpoles. Boycott (1988) made a personal observation: "The number of tooth-rows depends on the age of the tadpoles". In this study, tadpoles of four species of ghost frogs were examined to investigate and document the pattern of ontogenetic increase in labial tooth rows in Heleophrynidae in relationship to body size.

Two hundred and thirty one tadpoles of four species of ghost frogs were examined: *Heleophryne purcelli* (n = 36), *Heleophryne regis* (n = 77), *Heleophryne hewitti* (n = 75) and *Hadromophryne natalensis* (n = 43). New material was collected by lifting rocks in streams, with a standard aquarium net suspended below to catch dislodged tadpoles. Tadpoles were euthanized with tricaine methanesulfonate (MS222) solution and preserved in 10% buffered formalin. Voucher specimens are all hosted in the Port Elizabeth Museum (PEM; Appendix A), apart from a few tadpoles of *H. hewitti* that were measured, photographed and examined in the field. Tadpoles identifications are based on known distributions of respective species (Minter et al., 2004). Body length (tip of snout to the anterior part of the vent) represented overall size, and was measured using a Mitutoyo caliper (accuracy of 0.05 mm) and rounded off to the nearest 0.1 mm. As the number

Table 1. Summary table indicating body length, number of posterior labial tooth rows, correlation between the two characters of southern African Ghost frog tadpoles.

	Body length (mm)	Number of posterior labial tooth rows	Spearman Rank-Order Correlation (R)	Two-tailed value (P)
Heleophryne				
H. purcelli (n = 36)	15.6±4.07	7 to 17	0.34194	0.04437
H. regis (n = 77)	13.2±3.57	6 to 16	0.5429	0
H. hewitti (n = 75)	13.5±3.57	5 to 14	0.48837	1E-05
Hadromophryne				
H. natalensis (n = 43)	18.8±3.26	13 to 16	0.64413	0

of anterior labial tooth rows is constant for all four species, only the posterior labial tooth rows with visible teeth were counted with the aid of a Nikon SMZ1270 dissecting microscope, or a hand lens in field observations. Due to environmental conditions or the effect of high amphibian chytrid infections in Heleophrynidae (Tarrant et al., 2013) some labial teeth get damaged. Tadpoles belonging to *Heleophryne* and *Hadromophryne* cannot be accurately staged (Gosner, 1960), as the hind limb buds, used in standard tadpole staging, develop in a small pouch. Data analysis was carried out with Program R (R Development Core Team, 2014) and summarised in Table 1. Spearman rank-order correlation analysis was run with only body length and the number of posterior labial tooth rows.

The number of posterior labial tooth rows varied from 5-17. It is also noted that the posterior labial tooth rows can be divided into two groups: primary posterior labial tooth rows and secondary posterior labial tooth rows (Fig. 2). Primary posterior labial tooth rows are characterised by being well developed, evenly spaced, continuous and darkly pigmented; they ranged between 4-6 (mainly 5) rows. Secondary posterior labial tooth rows varied from 1-10: small, broken, narrowly spaced rows that fade away posteriorly. A strong positive correlation existed between body length and the number of posterior labial tooth rows in all four species and is statistical significant (Table 1 and Fig. 3). Correlation data indicates that the body length explains 34-64% of the variation in number of posterior labial tooth rows. The strongest correlation existed within *Had. natalenis* (R = 0.64; P = 0) and the lowest for *Hel. purcelli* (R = 0.34; P = 0.04). In general, a strong support exists to indicate that number of posterior labial tooth rows increase with body size. Remaining variations are due to environmental factors, such as feeding behaviour, food availability, flooding, and damage by the amphibian chytrid fungus (Smith et al., 2007).

Visser (1985) recorded a beak with fang-like teeth that precedes the tooth rows during the first month of development, though he only recorded the total length of tadpoles. Body length is more reliable than total length, as the tail could have been damaged and adds an additional unnecessary variable. His measurements thus had to be extrapolated to be able to include his data into this paper, achieved by multiplying Visser (1985) total length measurements with a factor of 0.38342. This factor was derived by dividing body length with the total length of the tadpoles studied in this paper, and applied to Visser's data (Table 2). The shaded area in Fig. 3 represented the period of fang-liked teeth described by Visser (1985).

The lack of data for specimens smaller than 7 mm (body length) in museum collections indicates that these tadpoles make use of an alternative microhabitat during early developmental stages and thus elude capture by researchers. Two years of bimonthly monitoring of tadpole numbers in the Elandsberg Mountains failed in detecting tadpoles smaller than 7 mm in main currents (W. Conradie pers. comm.). It was also reported by Visser (1985) that early developmental tadpoles are found between aggregations of small rocks where they are protected from the stronger currents. Visser (1985) found no support that the early beak and fang-like teeth are used for grasping and indications are that they are solely used for feeding, however the sucker develops before these structures and thus fulfils the role of attachment from hatching to avoid getting swept away. The true function of this fang-like teeth needs further investigation and could fill the same function as for *Leptodactylon* spp. tadpoles, in acting as a sieve to pick up detritus or preventing larger gravel/sand through (Mapouyat et al., 2014). Visser (1985) further hypothesised that the loss of the beak and fang-like teeth enables the tadpoles to switch between particular food sources, supported by the description and figure included in his paper, showing a tadpole adapted for feeding on detritus. These food sources are expected to be mostly available in the gullies or slow flowing sections of mountain streams close to where eggs would have been deposited, thus restricting the developing tadpoles to these habitats for the first few

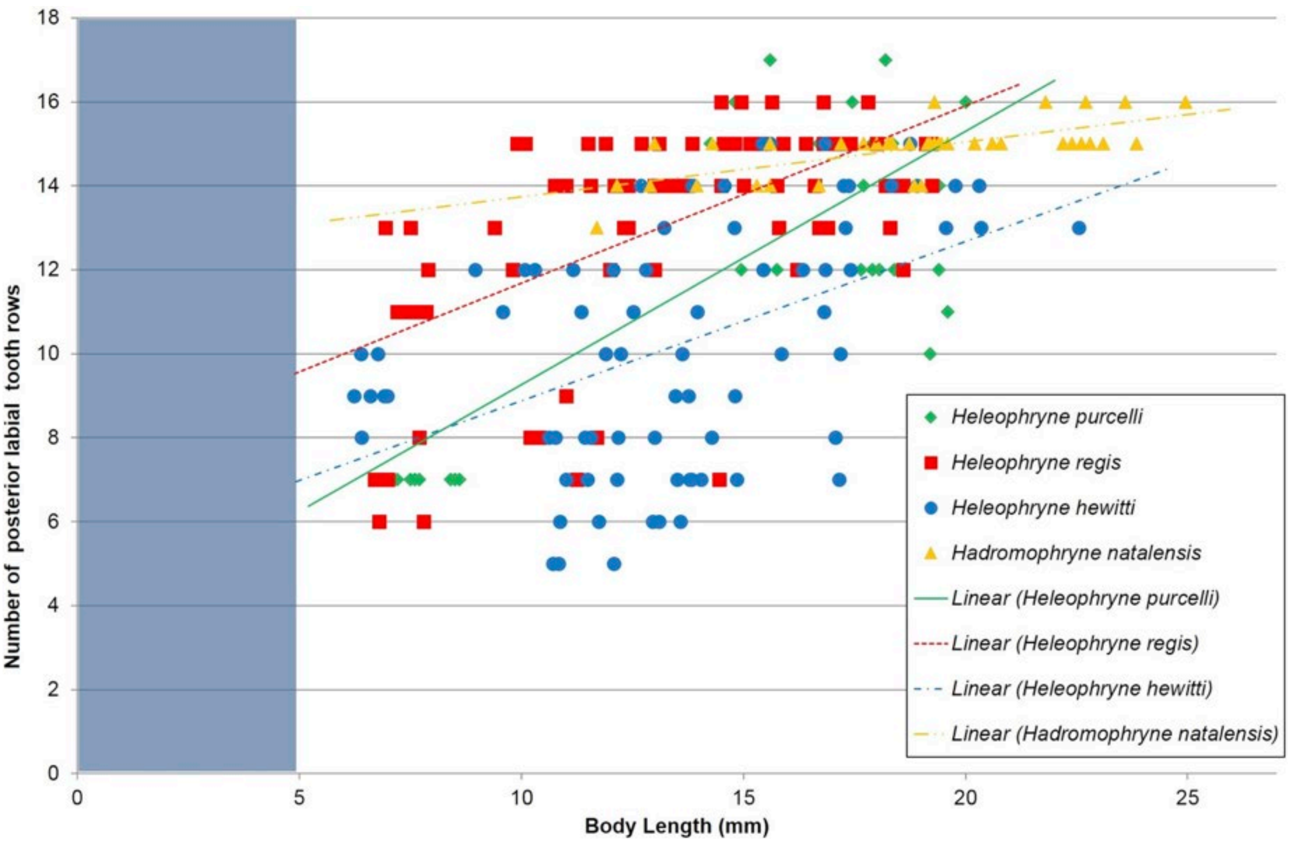

Fig. 3. Graph showing the relationship between the number of posterior labial tooth rows and body length (mm) of four species of Ghost frog tadpoles. The lack of data in the shaded area are explained by Visser (1985) description of fang-like teeth development (see main text)

Table 2. Early development of tadpole labial teeth according to Visser (1985): * body length was extrapolated by multiplying by a factor of 0.38342 and inserted into the table as additional data.

Day	Average total length (mm)	Average body length* (mm)	Description of labial teeth development
1	11.27	4.32	hatchling, no teeth
8 to 10	12.5	4.79	first fang-like teeth develop above
11 to 13	13.7	5.25	single row of fang-like teeth with 15-19 above and 2-6 below
14	13.8	5.29	20-26 fang-like teeth above; 6-8 fang-like teeth on each aspect below
20 to 22	15.95	6.12	1st and 2nd continuous labial tooth rows develop below fang-like teeth; fang-like teeth start to drop out
<30	16.54	6.34	4 labial tooth rows above and below; fang-like teeth all gone

months. It can't be disregarded that a smaller suctorial mouth and weaker suction power will further limit smaller tadpoles to this microhabitat. As the tadpoles develop additional rows of teeth, their feeding behaviour moves to scraping algae from rock surfaces, thus subsequently moving into faster flowing water as the suction ability of the suctorial mouth increases. With development, the number of labial tooth rows increases to allow for a maximum surface area for this feeding mechanism. In order to expand and enhance our knowledge of heleophrynid tadpoles, their ecology, especially feeding behaviour, still needs to be studied further.

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REFERENCES

Altig, R.A., McDiarmid, R.W. (1999): Body plan: development and morphology. In: McDiarmid, R.W., Altig, R. (eds.), Tadpoles: The Biology of Anuran Larvae, University of Chicago Press. Chicago, pp. 24-51.

Biju, S. D., Bossuyt, F. (2003): New frog family from India reveals an ancient biogeographical link with Seychelles. Nature **425**: 711-714.

Boycott, R.C. (1972): Ghost Frog eggs. Afr. Wildl. **26**: 43.

Boycott, R.C. (1988): Description of a new species of *Heleophryne* Sclater, 1899 from the Cape Province, South Africa (Anura: Heleophrynidae). Ann. Cape Prov. Mus. (Nat. Hist.). **16**: 309-319.

Boycott, R.C. (1999): In search of the elusive Ghost Frogs. In: Harisson, J.A., Burger, M., (eds.), Frogs and Frog Atlasing in Southern Africa. ADU Guide 4. Avian Demography Unit, University of Cape Town. Cape Town, pp. 59-63.

Boycott, R.C. (2004): Genus *Heleophryne* Sclater, 1898 (Family Heleophrynidae). In: Minter, L.R., Burger, M., Harrison, J.A., Braack, H.H., Bishop, P.J., Kloepfer, D., (eds). Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland. First Edition. Washington, Smithsonian.

Boycott, R.C., De Villiers, A.L. (1986): The status of *Heleophryne rosei* Hewitt (Anura: Leptodactylidae) on Table Mountain and recommendations for its conservation. S. Afr. J. Wildl. Res. **16**: 129-134.

Channing, A. (2001): Amphibians of Central and Southern Africa. Cornell University Press, Ithaca and London.

Du Preez, L.H., Carruthers, V.C. (2009): A Complete Guide to the Frogs of Southern Africa. Cape Town, Struik Nature.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., Desa̓, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, L.B., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C. (2006): The amphibian tree of life. Bull. Am. Mus. Nat. Hist. 297. New York, pp. 370.

Gosner, K.L. (1960): A simplified table to stage anuran embryos and larvae with notes on identification. Herpetologica **6**: 183-190.

Hewitt, J. (1913): Description of *Heleophryne natalensis*, a new batrachian from Natal; and notes on several South African batrachiens and reptiles. Ann. S. Afri. Mus. **2**: 475-484.

IUCN (2010): The IUCN Red List of Threatened Species. Version 2015.2 <www.iucnredlist.org>. Downloaded on 6 July 2015.

Khan, M.S., Mufti, S.A. (1994): Oral disc morphology of amphibian tadpole and its functional correlates. Pak. J. Zoo. **26**: 25-30.

Mapouyat, L., Hirschfeld, M., Rödel, M.-O., Liedtke, H.C., Loader, S.P., Gonwouo, L.N., Dahmen, M., Doherty-Bone T., Barej M.F. (2014): The tadpoles of nine Cameroonian *Leptodactylodon* species (Amphibia, Anura, Arthroleptidae). Zootaxa **3765**: 29-53.

Minter, L.R., Burger, M., Harrison, J.A., Braack, H.H., Bishop, P.J. & Kloepfer, D., Ed. (2004): Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland. First Edition. Washington, Smithsonian.

Passmore, N.I., Carruthers, V.C. (1995): South African Frogs, 2ed. Southern Book Publishers and Witwatersrand University Press, Johannesburg.

R Development Core Team. (2013): R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria. Available from: <http://www.R-project.org>.

Rose, W. (1926): Some field notes on the Batrachia of the Cape Peninsula. Ann. S. Afr. Mus. **20**: 433-450.

Rose, W. (1950): The Reptiles and Amphibians of Southern Africa. Maskew Miller, Cape Town.

San Mauro, D., Vences, M., Alcobendas, M., Zardoya, R., Meyer, A. (2005): Initial diversification of living amphibians predated the breakup of Pangaea. Am. Nat. **165**: 590-599.

Smith, K.G., Weldon, C., Conradie, W., Du Preez, L.H. (2007): Relationships among size, development, and *Batrachochytrium dendrobatidis* infection in African tadpoles. Dis. Aquat. Org. **74**: 159-164.

Tarrant, J., Cilliers, D., Du Preez, L.H., Weldon, C. (2013): Spatial assessment of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in South Africa confirms endemic and widespread infection. *PLoS ONE* **8(7)**: e69591. doi:10.1371/journal.pone.0069591.

Van Dijk, D.E. (1966): Systematic and field key to the families, genera and described species of southern African Anuran tadpoles. Ann. Natal Mus. **18**: 231-286.

Van Dijk, D.E. (2008): Clades in Heleophrynid Salientia. Afr. J. Herp. **57**: 43-48.

Venesky, M.D., Wassersug, R.J., Jorgensen, R.M., Parris, M.J. (2011): Comparative feeding kinematics of temperate pond-dwelling tadpoles (Anura, Amphibia). Zoomorphology **130**: 31-38.

Venesky, M.D., Wassersug, R.J., Parris, M.J. (2010a): How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenocephala*)? Biol. Bull. **218**:160-168.

Venesky, M.D., Wassersug, R.J., Parris. M.J. (2010b): The impact of variation in labial teeth on the feeding kinematics of Southern Leopard Frog (*Lithobates sphenocephala*) tadpoles. Copeia **2010**: 481-486.

Visser, J.D. (1971): Hunting the eggs of the ghost frog (*Heleophryne purcelli orientalis* FitzSimons). Afr. Wildl. **25**: 22-24.

Visser, J.D. (1985): The fang-like teeth of the early larvae of some *Heleophryne* (Anura: Leptodactylidae). S. Afr. J. Sci. **81**: 200-202.

Wager, V.A. (1965): The Frogs of South Africa. Purnell, Cape Town.

Appendix A

List of material examined, 'PEM T' refers to the tadpole collection of the Port Elizabeth Museum and the numbers in brackets refer to the number of tadpoles in each lot:

Heleophryne purcelli: PEM T275 (4), PEM T277 (11), PEM T278 (19), PEM T285 (2).

Heleophryne regis: PEM T30 (8), PEM T34 (20), PEM T36 (4), PEM T284 (9), PEM T286 (11), PEM T287 (2), PEM T288 (18), PEM T420 (5).

Heleophryne hewitti: PEM T327 (2), PEM T408 (7), PEM T373 (2), PEM T419 (5), PEM T694 (4), PEM T694 (6), 49 field observations.

Hadromophryne natalensis: PEM T82 (24), PEM T214 (16), PEM T274 (1), PEM T301 (2).